JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH

How to cite this article:
BABAEI P , RAHMANI-NIA F, NAKHOSTIN B, BOHLOOLI S H.THE EFFECT OF VC ON IMMUNOENDOCRINE AND OXIDATIVE STRESS RESPONSES TO EXERCISE.Journal of Clinical and Diagnostic Research [serial online] 2009 August [cited: 2009 August 7]; 3:1627-1632.
Available from
http://www.jcdr.net/back_issues.asp?issn=0973-709x&year=2009&month=August&volume=3&issue=4&page=1627-1632&id=432

ORIGINAL ARTICLE

The Effect Of VC On Immunoendocrine And Oxidative Stress Responses To Exercise

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ABSTRACT

The depression of the immune system function that is typically observed after strenuous exercise is believed to be possibly mediated by stress hormones, cytokines and oxidative stress. The aim of this study was to measure immunoendocrine and oxidative stress responses after the ingestion of two different doses of Vitamin C (VC) supplementation. Twenty-four healthy untrained males participated in a 30-min exercise at 75%Vo2max. Immediately pre-exercise, the participants received either of the following regimens: placebo, 500 mg and 1000 mg of VC. Blood samples were obtained prior to ingestion, immediately after ingestion, 2hrs after ingestion and also 2hrs and 24hrs after exercise.

Vitamin C used in doses of 500 mg and 1000 mg could significantly increase the plasma VC concentration and antioxidant capacity in both vitamin receiving groups. The increase in total antioxidant capacity (TAC) followed a significant decrease in post-exercise oxidative stress markers like malondialdehyde (MDA) ($P<0.05$). Markers of inflammation (total leukocytes, neutrophils and IL-6), muscle damage, creatine kinase (CK) and stress hormone (cortisol) were found to significantly increase in response to the exercise ($P<0.05$), but VC supplementation failed to decrease these factors significantly. The results suggest that acute supplementation with moderate and high doses of VC might prevent exercise-induced lipid peroxidation but not inflammatory markers.

Key Words: Ascorbate; stress hormone; cytokine; oxidative stress

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Introduction

It has been documented that high intensity exercise not only induces oxidative stress, but also elicits the mobilization and functional augmentation of neutrophils and monocytes. The changes in the immunoendocrine system and also the paracrine secretion of cytokines lead to the suppression of cellular immunity and increased the susceptibility to infections. Cytokines are considered to induce systemic bioactivity following exercise as anti-inflammatory and also proinflammatory substances [1]. It has been known that physical exercise is a model of stress increase energy demand to a large extent, and subsequently oxygen uptake [2],[3]. Muscle damage subsequent to exercise can cause inflammation and release of superoxides and free radicals, resulting in lipid peroxidation [4],[5],[6]. Reactive oxygen species (ROS) "leaking" from the mitochondria during exercise are considered as a main source of oxidative stress [3],[5]. Oxidative stress may result from oxidative reactions within the skeletal muscle [7]. The majority of free radicals produced in vivo are oxidants which are capable of oxidizing a range of biological molecules including carbohydrates, amino acids and fatty acids. Moreover, exercise induces highly stereotyped changes in leukocyte subpopulations [8]. Immune cells are mobilized and activated during exercise in response to muscle damage and also via the actions of stress hormones (catecholamines, growth hormone, cortisol) that are released in

response to increasing metabolic demands and core temperature during exercise [9],[10]. Immunological studies revealed that a range of antioxidant defenses have evolved in the body. The main nonenzymatic antioxidants include VC and E. The antioxidant defenses of the body are usually adequate to prevent substantial tissue damage, whereas the stress situation in which there is imbalance could lead to deleterious effects [11].Vitamin C is able to protect endogenous lipids from detectable oxidative damage induced by aqueous peroxyl radicals and other reactive oxygen species [12]. Previous studies investigating the protective effects of supplementation with VC have been inconclusive: inhibition of lipid peroxidation [13],[14], no effect [15],[16], and even increased lipid peroxidation [17]. Since, VC is water-soluble it´s availability may be adequate after a single dose usage, and hypothetically there may be no need for prolonged supplementation. The aim of this study was to measure the immunoendocrine and oxidative stress responses after the ingestion of two different doses of VC, high and moderate dose, before exercise, in untrained men participating in a 30-min run at 75% Vo_{2max}.

Material and Methods

Twenty-four untrained male students volunteered to take part in this study, which had approval from the Guilan University Ethical Advisory Committee. All subjects were informed verbally and in writing about the nature and demands of the study, and subsequently completed a health history questionnaire and informed consent. Participants with smoking habits, vegetarians and those who took vitamin supplements were excluded from the study and were allocated to 3 groups in a single blind design: those on high dose VC (HD), those on moderate dose VC (MD) or those on placebo (P) [Table/Fig 1]. They performed two preliminary treadmill-based tests at least 10 days prior to the main trial. Briefly, a Bruce test to determine Vo_{2max} and also an incremental submaximal running test to determine the relationship between running speed and oxygen uptake were taken.

(Table/Fig 1) Subjects characteristics in placebo (P), moderate(MD), and high dose (HD)groups.

Groups	Age (years)	Height (cm)	Body mass (kg)	BMI ($kg.m^2$)	Vo_{2max} ($ml.kg^{-1}.min^{-1}$)	Skin folds (mm)
P	22.1±0.6	174.1±1.8	72.4±3.1	23.8±0.8	39.1±1.6	49.6±5.8
MD	21.5±0.8	173.1±5.8	68.4±10.3	21.5±1.0	40.6±4.7	43.9±5.7
HD	20.9±0.7	176.4±2.3	67.4±4.4	21.5±1.0	39.3±1.9	37.4±3.6

Values for each group represent means ± SEM (n=8)

Experimental Design And Procedure

On the day of the test, participants received either of the following regimens: placebo, 500 mg and 1000 mg of VC with a carbohydrate free breakfast. After a 10-min warm-up consisting of running at 50% Vo_{2max} (5-min) and stretching (5-min), participants ran on the treadmill for 30-min at 75% $Vo_{2max.}$ Blood samples were taken immediately after exercise and 2hrs and 24hrs after exercise. Plasma and serum were obtained using standard procedures. Two small aliquots of EDTA-treated blood were removed for the determination of differential leukocytes using a Cell counter (K-1000 Sysmax, Japan). Due to VC analysis, 0.03 ml distilled water and 0.06 ml of 10% metaphosphoric acid were added to 0.03 ml of plasma (Merck, Germany) and was vortexed in a 1.5-ml centrifuged tube for ~ 10s. The suspension was placed over ice for at least 10 min and was sheltered from strong light. Then, the mixture was centrifuged at 23000 g for 10 min at 4˚C and was infused to an HPLC[1] column (Jasco, Japan) in 0.05 volume using a Hamilton syringe [18].

In order to analyze MDA, an aliquoted portion of 0.05 ml serum was added to 0.25 ml of 0.1 M Trichloroacetic acid (TCA) and 0.7 ml distilled water and the samples were centrifuged at 4500 g for 5 min and were used for HPLC analysis [19]. Serum Creatine kinase (CK) was determined using commercially available methods (autoanalizer, Roche Hittachi-911, Germany and Japan) and IL-6 was analyzed using ELISA (Dynex, USA). Serum cortisol was measured by electrochemiliuminescence (Roche Hittachi, Germany and Japan).

Statistical Analysis

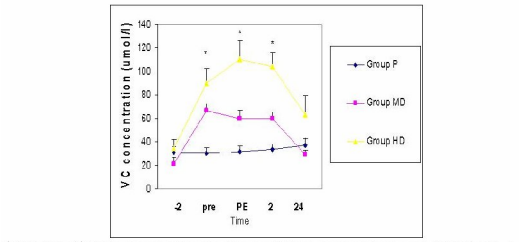
Results are expressed as means ± SEM, and $p<0.05$ was considered to be statistically significant. An independent two-way analysis of variance with repeated measures and the

1. High Performance Liquid Chromatography

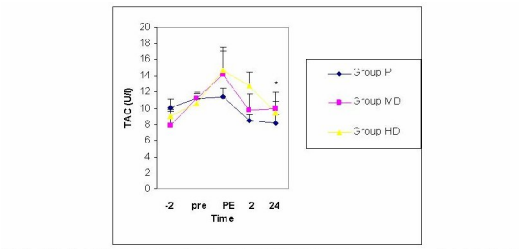
Tukey Honest post test were used to compare results between treatments and over time. When there were only single comparisons, the student's t-test with Bonferroni correction for correlated data was used to determine whether any differences between treatments existed.

Results

The baseline resting plasma VC concentration was not different between groups [Table/Fig 2]. Two hours after supplementation, plasma VC was found to be significantly elevated in the HD and MD groups ($p<0.05$) and decreased over the course of exercise, but was still significant immediately and 2hrs after exercise in both groups ($p<0.05$). Then, it returned to baseline levels twenty-four hours after exercise. Baseline serum TAC was not different between groups [Table/Fig 3]. The total antioxidant capacity increased immediately after exercise in the placebo group ($p>0.05$) and decreased 2hrs and 24hrs after exercise even on comparison with the baseline values ($p<0.05$). In the MD and HD groups, TAC increased after supplementation and continued immediately after exercise and 2hrs later, returning to baseline values after 24hrs. There were no significant differences between the three treatment groups ($p>0.05$).



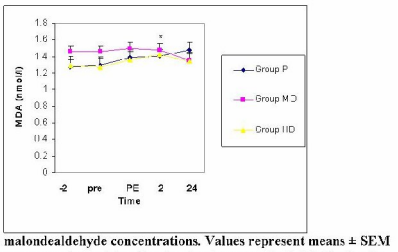
(Table/Fig 2) VC concentrations in plasma. Values represent means ± SEM (n=8). * significantly above baseline and placebo values ($p<0.05$). P: placebo, MD: moderate dose and HD: high dose -2: baseline, PE: post-exercise.



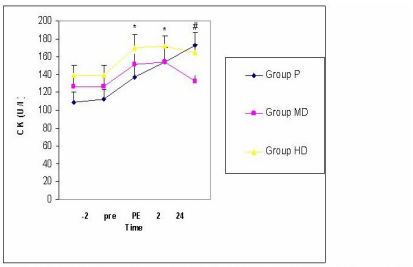
(Table/Fig 3) TAC concentrations in plasma. Values represent means ± SEM (n=8). * significantly decline in comparison with pre-exercise ($p<0.05$). P: placebo, MD: moderate dose and HD: high dose -2: baseline, PE: post-exercise.

Markers Of Lipid Peroxidation And Muscle Damage

Blood MDA is shown in [Table/Fig 4]. MDA increased 2hrs after exercise only in the placebo group ($p<0.05$). There were no significant differences between the treatment groups for MDA over the course of exercise ($p<0.05$).



(Table/Fig 4) Serum malondealdehyde concentrations. Values represent means ± SEM (n=8). * Values above pre-exercise and baseline in placebo group ($p<0.05$). P: placebo, MD: moderate dose and HD: high dose -2: baseline, PE: post-exercise.



(Table/Fig 5) Serum CK before and after exercise. Values represent means ± SEM (n=8). * Values in all groups above pre-exercise ($p<0.05$). # Values in placebo group above pre-exercise ($p<0.05$). P: placebo, MD: moderate dose and HD: high dose -2: baseline, PE: post-exercise.

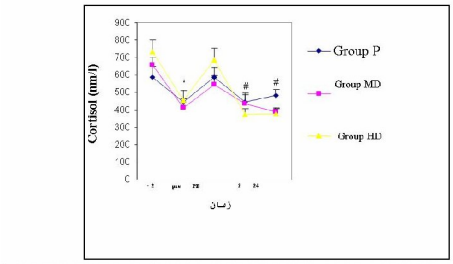
The blood CK concentration is shown in [Table/Fig 5]. CK increased above baseline values after exercise in all groups. The increase of CK was significant immediately and 2hrs after exercise in all groups as well as 24hrs after exercise only in the placebo group ($p< 0.05$). There were no differences among the groups for CK over the course of the experiment ($p>0.05$).

Serum cortisol is shown in [Table/Fig 6]. Cortisol concentrations increased immediately after exercise in both the groups ($p<0.05$). Then, serum cortisol concentrations declined to almost pre-exercise levels, 2hrs and 24hrs after exercise ($p>0.05$). There were no significant differences between the cortisol concentrations in the placebo and the VC groups ($p>0.05$).

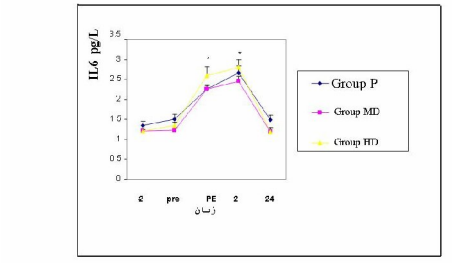
IL-6 concentrations were elevated after exercise ($p<0.05$) and declined to almost pre-exercise levels at 24 hrs after exercise in both groups. There were no detectable differences

between the placebo and the VC groups ($p>0.05$). Serum IL-6 is shown in [Table/Fig 7].

The effect of 30 min of exercise at 75% Vo_{2max} on circulating total leukocyte, neutrophil and lymphocyte counts are shown in [Table/Fig 8]. There were no significant differences in lymphocyte counts in both groups after the exercise was compared to pre-exercise ($p>0.05$). Also, there was no significant difference between the groups for total leukocyte, neutrophil and lymphocyte counts over the course of the experiment ($p>0.05$).



(Table/Fig 6) Plasma cortisol concentrations before and after exercise. Values represent means ± SEM (n=8). VC: VC, P: placebo, -2: baseline, PE: post-exercise.



(Table/Fig 7) Serum IL-6 concentrations. Values represent means ± SEM (n=8). * Values in placebo and VC groups above baseline and pre-exercise ($p<0.05$). VC: VC, P: placebo, -2: baseline, PE: post-exercise.

(Table/Fig 8) Blood Leukocyte subpopulations in placebo (P), moderate (MD), and high dose (HD) groups. Values for each group represent means (SEM) (n=8)

Variable \ Time	Groups	Baseline(-2)	Pre-exercise	Post-exercise	1h	24h
WBC (/µl)	P	6450 ± 742	6553 ± 769	7729 ± 860	10713 ± 864*	6030 ± 756
	VC(1000)	6300-469	7100+589	8830±624*	9829±826*	6529±581*
	VC(500)	6038 ± 323	6938 ± 445	8188 ± 554*	9913 ± 756*	5700 ± 392
Neutrophils(/µl)	P	3507 ± 433	4440 ± 720	5161 ± 658*	8267 ± 751*	3015 ± 376
	VC(1000)	3438+367	4669±434	5752±490	7300±973	3747±433
	VC(500)	3197 ± 233	4149 ± 347	4835 ± 356	7483 ± 701	3226 ± 326
Lymphocytes(/µl)	P	2353 ± 247	1650 ± 119	2077 ± 216	1918 ± 144	2022 ± 107
	VC(1000)	2473±288	1940±213	2492±178	2230±186	2008±507
	VC(500)	2281 ± 295	1754 ± 257	2523 ± 440	1883 ± 255	1950 ± 225

Values represent means ± SEM (n=8). * Values in placebo and VC groups above baseline and pre-exercise ($p<0.05$).

Discussion

The main purpose of this study was to investigate whether acute supplementation with high and moderate doses of VC would have an effect on the inflammation and lipid peroxidation factors induced by physical stress. Acute supplementation of VC with both doses of 500 mg and 1000 mg could increase plasma VC levels 2hrs after supplementation. Total antioxidant capacity decreased significantly 24hrs after exercise as compared to pre-exercise in the placebo group, proving the effect of VC supplementation as a putative antioxidant. It has been known that exercise by itself could increase plasma VC. This increase relates to the elevation of cortisol during exercise, which promotes the efflux of ascorbic acid from the adrenal gland [20],[21] or the mobilization of ascorbic acid from other tissues such as leukocytes and erythrocytes [20]. Contrary to some studies [16],[22], plasma VC concentration was not elevated in the placebo group after exercise. This result is most likely, because of lack of considerable change in serum cortisol. One of the peroxidation factors, MDA, was significantly blunted after exercise in both VC supplemented groups (MD an HD), whereas in the placebo group, MDA increased significantly 2hrs after exercise. The result of our study is in agreement with Ashton et al., but not with Thompson et al. and Davidson et al. The effect of VC on MDA possibly depends on the fitness level or training status of the participants [25]. It is assumed that responses to antioxidant supplementation in untrained individuals are much more than in endurance-trained athletes. Some studies (Miyazaki et al., and Fatouros et al.), in contrary to others (Tiidus et al. and Tonkonogi et al.), reported that endurance training could improve the endogenous antioxidant defenses. Moreover, differences in the modes, duration, and intensity of exercise, as well as variation in the methodologies used to assess lipid peroxidation, could be the possible reasons of inconsistencies. The marker of muscle damage (CK) increased immediately after exercise and continued two hours later and returned to pre-exercise values after 24 hours in both groups. The efflux of this enzyme was not different between the VC and the placebo groups. It seems that acute supplementation with VC had no effect on CK as a muscle damage marker. According to Feasson et al., the increase in CK may be due to disruption of the muscle fiber structures, and consequently due to leakage of this protein into the circulation. This efflux relates to increase in the ROS- induced membrane permeability of the muscle cells [31], [32], [33]. Peake et al. and Kobayashi et al., have reported that after exercise, CK

reaches its peak in 24 or 48hrs, whereas in our study, after the similar time course, CK returned to baseline levels in VC supplemented groups. The reduction in CK relates most likely to blunting MDA by VC pretreatment. However, the low duration and intensity of the exercise used in our study is the reason for insignificant changes. In our study, IL-6 was enhanced only two times in both groups. The insignificant change in IL6 is in agreement with Davidson et al, Davison and Gleeson, but not Thompson et al. According to Paczek et al., the change in IL-6 mainly depends on energy expenditure, calorie intake, glycogen demand and the duration and intensity of exercise. In the present study, VC could not affect IL-6, probably because of the short time of muscles involvement in performance, which was not sufficient to provide the optimum uptake of VC into cytokine producing tissues and to induce molecular cascades of IL6 production [22]. On the other hand, another humeral factor such as blood glucose levels can regulate changes in IL-6 and cortisol during exercise [36]. The lack of significant effects in WBC counts, could probably relate to insignificant changes in IL6 and cortisol too. It has been known that muscle-derived IL6 has a role in exercise induced leukocyte trafficking directly and indirectly by cortisol elevation.

In summary, acute supplementation with both doses of VC, 2hrs before exercise increased plasma concentrations of this vitamin and consequently could alleviate lipid peroxidation and muscle damage induced by physical stress. Therefore, VC supplementation prevented oxidative damage but had no apparent effect on inflammation, indicating different cascades of oxidative damage and inflammation. It can be conclude that intake of a moderate dose of VC, as an useful antioxidant could protect body from oxidant stress and is of benefit to physical activity. Future studies could be designed to measure the complete profile of inflammation and ROS induced by exercise in different time intervals after VC intake.

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